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(57) Abstract

Linear octapeptide analogs of somatostatin which inhibit secretion of growth hormone.

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LINEAR SOMATOSTATIN ANALOGS

Background of the Invention

This invention relates to therapeutic peptides.

A number of somatostatin analogs exhibiting Growth Hormone-releasing-inhibiting activity have been described in the literature, including analogs containing fewer than the naturally occurring fourteen amino acids. For example, Coy et al. U.S. Patent No. 4,485,101, hereby incorporated by reference, describes dodecapeptides having an N-terminal acetyl group, a C-terminal NH_2, D-Trp at position 6, and p-Cl-Phe at position 4. (Herein, when no designation of configuration is given, the L-isomer is intended.)

Abbreviations: Nle = norleucine, Nal - naphthylalanine

Summary of the Invention

In general, the invention features a linear somatostatin analog of the formula:

$$\begin{array}{l} R_1 \diagdown \\ \quad\quad A^1 - A^2 - A^3 - \text{D-Trp} - \text{Lys} - \\ R_2 \diagup \quad\quad\quad A^6 - A^7 - A^8 - R_3 \end{array}$$

wherein

A^1 is a D-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = H, CH_3,

Cl, Br, F, OH, OCH_3, NO_2), p-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, NO_2), 2,4-dichloro-Phe, pentafluoro-Phe;

A^2 is any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, NO_2), p-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

A^3 is any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, Trp, Tyr, ß-Nal, o-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, NO_2), p-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

A^6 is any of Ala, pyridyl-Ala, Leu, Ile, Val, Lys, Met, Nle, Thr, Trp, Ser, ß-Nal, o-X-Phe (wherein X = CH_3, Cl, Br, F, OH, OCH_3, NO_2), p-X-Phe (wherein X = CH_3, Cl, Br, F, OH, OCH_3, NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

A^7 is any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, NO_2), p-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

A^8 is a D- or L-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Ser, Thr, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein

$X = CH_3$, Cl, Br, F, OH, OCH_3, NO_2), p-X-Phe (wherein $X = CH_3$, Cl, Br, F, OH, OCH_3, NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

each R_1 and R_2, independently, is H, lower (1-5 carbon atoms) acyl, or lower alkyl; and R_3 is H, NH_2, or lower alkyl; provided that at least one of A^1 and A^8 must be an aromatic amino acid; and further provided that if either A^2 or A^7 is an aromatic amino acid, then A^8 cannot be an aromatic amino acid; and further provided that R_4 may be nothing or may be a carbohydrate, e.g., $C_x(H_20)_y$, where x is 1-18 and y is 1-16, linked through the hydroxyl group of Ser or Thr; or a pharmaceutically acceptable salt thereof. The linkage of the carbohydrate group to the serine or threonine hydroxyl group may be an alpha or beta linkage.

R_4 may, for example, be a protected glycosyl radical, e.g., a glucofuranosyl or glucopyranosyl radical which is derived from naturally occurring aldetetroses, aldopentoses, aldohexoses, ketopentoses, deoxyaldoses, aminoaldoses and oligosaccharides such as di- and trisaccharides, and stereoisomers thereof. R_4 may be derived from natural D- or L-monosaccharides which occur in microorganisms, plants, animals r humans, such as ribose, arabinose, xylose, Lyxose, allose, altrose, glucose, mannose, gulose, idose, glactose, talose, erythose, threose, psicose, fructose, sorbose, tagatose, xylulose, fucose, rhamnose, olivose, oliose,

mycarose, rhodosamine, N-acetyl-glucosamine, N-acetylgalactosamine, n-acetyl-m,annosamine, or disaccharides such as maltose, lactose, cellobiose, gentiobiose, N-acetyl-lactosamine, chitobiose, ß-galactopyranosyl-(1,3)-N-acetylgalactosamine and ß-galactopyranosyl(1,4)-N-acetyl-glucosamine, and the synthetic derivatives thereof, such as 2-deoxy-, 2-amino, 2-acetamido- or 2-halogeno-, especially bromo- and iodo- sugars.

Protective groups may be, for example, the (C_1-C_{10})-acyl groups, such as (C_1-C_6)-alkanoyl (e.g., acetyl, trichloroacetyl, trifluoroacetyl), benzoyl or p-nitrobenzoyl, and optionally modified methyl, methyloxymethyl, benzyl, tetrahydropyranyl, benzylidene, isopropylidene or trityl group, or the acyl protective groups, e.g., acetyl.

Preferably, of A^1 and A^2, only one is an aromatic amino acid; and of A^7 and A^8, only one is an aromatic amino acid.

In preferred embodiments A^1 is a D-isomer of any of Trp, ß-Nal, o-X-Phe (wherein X = CH_3 or OCH_3), p-X-Phe (wherein X = CH_3 or OCH_3) and A^8 is a D- or L-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Ser, Thr, Val, Met, Nle, o-X-Phe (wherein X = Cl, Br, F, OH, NO_2), p-X-Phe (wherein X = Cl, Br, F, OH, NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe.

In other preferred embodiments A^1 is a D-isomer of

any of o-X-Phe (wherein X = H, Cl, Br, F, OH, or NO_2), p-X-Phe (wherein X = H, Cl, Br, F, OH, or NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe; and A^8 is a D- or L-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Thr, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = CH_3 or OCH_3), or p-X-Phe (wherein X = CH_3 or OCH_3).

In other preferred embodiments A^8 is a D- or L-isomer of any of Thr, Trp, ß-Nal, o-X-Phe (wherein X = CH_3 or OCH_3), or p-X-Phe (wherein X = CH_3 or OCH_3); and A_1 is Phe or a D-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, o-X-Phe (wherein X = H, Cl, Br, F, OH, NO_2), p-X-Phe (wherein x = H, Cl, Br, F, OH, NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe.

In other preferred embodiments A^8 is a D- or L-isomer of any of Ser, Thr, o-X-Phe (wherein X = Cl, Br, F, OH, or NO_2), p-X-Phe (wherein X = Cl, Br, F, OH, or NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe; and A^1 is a D-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = CH_3 or OCH_3), or p-X-Phe (wherein X = CH_3 or OCH_3).

More preferably, A^1 = ß-D-Nal or D-Phe; A^2 = Ala, Phe or p-chloro-Phe; A^3 = Tyr or Phe; A^6 = Val, Lys or Thr; A^7 = Ala or Phe; A^8 = Thr or D-ß-Nal.

Preferred compounds of the invention include D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;

D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;
D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2; and
D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-ß-D-Nal-NH_2.

In other preferred embodiments, a therapeutically effective amount of the therapeutic compound and a pharmaceutically acceptable carrier substance, e.g. magnesium carbonate, lactose, or a phospholipid with which the therapeutic compound can form a micelle, together form a therapeutic composition, e.g. a pill, tablet, capsule, or liquid for oral administration to a human patient, a spreadable cream, gel, lotion, or ointment to be applied topically or to be iontorphoretially forced through the skin of a human patient in need of the compound, a liquid capable of being administered nasally as drops or spray, or a liquid capable of intravenous, parenteral, subcutaneous, or intraperitoneal administration. The pill, tablet or capsule can be coated with a substance capable of protecting the composition from the gastric acid in the patient's stomach for a period of time sufficient to allow the composition to pass undisintegrated into the patient's small intestine. The therapeutic composition can also be in the form of a biodegradable or nonbiodegradable sustained release formulation for intramuscular administration. For maximum efficacy, zero order release is desired, and can be obtained using an implantable or external pump, e.g., Infusoid™ pump, to administer the therapeutic composition.

The compounds of the invention are active in inhibiting the secretion of growth hormone, somatomedins (e.g., IGF-1), insulin, glucagon, and other autoparacrine growth factors or pancreatic growth factors. The compounds of the invention are acyclic and, therefore, stable and resistant to oxidation. In addition, the acyclic nature of the peptide facilitates synthesis and purification, improving efficiency and reducing manufacturing costs.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

The drawings will first be described.

Drawings

Fig. 1 is a graph showing the effects of linear analogs on growth hormone secretion by rat pituitary cells.

Fig. 2 is a graph showing the effects of linear analogs on growth hormone secretion by rat pituitary cells.

Structure

The compounds of the invention have the general formula recited in the Summary of the Invention, above. They are all octapeptide analogs of somatostatin which have D-Trp at the fourth position and Lys at the fifth position. It has been found that p-chloro-phenylalanine at position A^2 and threonine at position A^8 are modifications which particularly

enhance activity. However, compounds containing an aromatic amino acid at position A^8 are inactive if there is an aromatic amino acid at either or both positions A^2 and A^7.

The compounds can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids such as the hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid.

Synthesis

The synthesis of one therapeutic peptide follows. Other peptides can be prepared by making appropriate modifications, within the ability of someone of ordinary skill in this field, of the following synthetic method.

The first step in the preparation of the peptide

D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2

is the preparation of the intermediate:

Boc-D-Phe-Phe-Phe-D-Trp-N-benzyloxycarbonyl-Lys-O-benzyl-Thr-Phe-O-benzyl-Thr-benzhydrylamine resin, as follows.

Benzhydrylamine-polystyrene resin (Advanced ChemTech, Inc.) (1.2g, 0.5 mmole) in the chloride ion form is placed in the reaction vessel of an Advanced ChemTech peptide synthesizer

programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 and 25 min each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; and (f) 10% triethylamine in chloroform.

The neutralized resin was stirred with Boc-O-benzyl-threonine and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 hr and the resulting amino acid resin is then cycled through steps (a) to (f) in the above wash program. The following amino acids (1.5 mmole) are then coupled successively by the same procedure:

Boc-Phe, Boc-O-benzyl-Thr, Boc-N-benzyloxycarbonyl-lysine, Boc-D-Trp, Boc-Phe, and Boc-Phe and Boc-D-Phe. After washing and drying, the completed resin weighed 1.70 g.

The resin (1.70 g, 0.5 mmole) is then mixed with cresol (5 ml), dithiothreitol (100 mg) and anhydrous hydrogen fluoride (35 ml) at 0°C and stirred for 45 min. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen, and free peptide precipitated and washed with ether. The crude peptide is then dissolved in a minimum volume of 50% acetic acid and eluted on a column (2.5 x 100 cm) of Sephadex G-25 using the same solvent. Fractions containing a major component by UV absorption and thin layer chromatography are then pooled, evaporated to a small volume and applied to a column (2.5 x 50 cm) of Vydac octadecylsilane silica (10-15

μM).

The column was eluted with a linear gradient of 10-45% acetonitrile in 0.1% trifluoroacetic acid in water. Fractions are examined by thin layer chromatography and analytical high performance liquid chromatography and pooled to give maximum purity. Repeated lyophilization of the solution from water gives 65 mg of the product as a white, fluffy powder.

The product was found to be homogeneous by hplc and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the octapeptide.

Other peptides of the invention are prepared in an analogous fashion to those described above.

Effects of linear somatostatin analogs on growth hormone secretion in cultured rat pituitary cell dispersion

Octapeptides of the invention are tested for inhibtion of growth hormone-releasing-activity using rat pituitary cells, as follows.

Anterior pituitaries from adult Charles River CD male rats (Wilmington, MA) weighing 200-250 g and housed under controlled conditions (lights on from 0500-1900 h), were dispersed and cultured using aseptic technique by modification of previously described methods (Hoefer et al., 1984, Mol. Cell. Endocrinol. 35:229; Ben-Jonathan et al., 1983, Methods Enzymol. 103:249; Heiman et al., 1985, Endocrinology 116:410).

Pituitaries were removed from decapitated rats, sectioned, and then placed into a siliconized, liquid scintillation vial containing 2 ml 0.2% trypsin (Worthington Biochemicals, Freehold, NJ) in sterile-filtered Krebs-Ringer bicarbonate buffer supplemented with 1% bovine serum albumin, 14mM glucose, modified Eagle medium (MEM) vitamin solution and MEM amino acids (Gibco Laboratories, Grand Island, NY) (KRBGA). All glassware was siliconized as described by Sayers et al., 1971, Endocrinology 88:1063. The fragments were incubated in a water bath for 35 min at 37°C with agitation. The vial contents then were poured into a scintillation vial containing 2 ml 0.1% DNase (Sigma Chemical Co., St. Louis, MO) in KRBGA and incubated for 2 min at 37°C with agitation. After incubation the tissue was decanted back into the centrifuge tube and allowed to settle. Medium was discarded, and pituitary sections were washed 3 times with 1 ml fresh KRBGA. The cells were then dispersed by gently drawing the fragments into and expelling them out of a siliconized, fire-polished Pasteur pipette in 2 ml 0.05% LBI (lima beam trypsin inhibitor, Worthington Biochemicals). Dispersed cells were filtered through a 630μm diameter Nylon mesh (Tetko, Elmsford, NY) into a fresh 15 ml centrifuge tube and harvested by centrifugation at 100 x g for 1 min. The final speed was attained gradually through a centrifugation period of 17 min.

After centrifugation, medium was discarded and the

pelleted cells were resuspended in fresh LBI (2 ml) with a Pasteur pipette. The dispersed cells were then diluted with approximately 15 ml sterile-filtered Dulbecco's modified Eagle medium (GIBCO), which was supplemented with 2.5% fetal calf serum (GIBCO), 3% horse serum (GIBCO), 10% fresh rat serum (stored on ice for no longer than 1 h) from the pituitary donors, 1% MEM nonessential amino acids (GIBCO), gentamycin (10 ng/ml; Sigma) and nyatatin (10,000 U/ml; GIBCO). The cells were poured into a 50 ml round-bottomed glass extraction flask with a large diameter opening and were counted with a lemacytometer (approximately 2,000,000 cells per pituitary) and randomly plated at a density of 200,000 cells per well (Co-star cluster 24; Rochester Scientific Co., Rochester, NY). The plated cells were maintained in the above Dulbecco's medium in a humidified atmosphere of 95% air and 5% CO_2 at 37°C for 96 h.

In preparation for a hormone challenge, the cells were washed 3x with medium 199 (GIBCO) to remove old medium and floating cells. Each dose of analog (diluted in normal saline in siliconized test tubes) was tested in the presence of 1 nM GRF(1-29)NH_2 (growth hormone releasing factor) in quadruplicate wells in a total volume of 1 ml medium 199 containing 1% BSA (fraction V; Sigma). After 3 h. at 37°C in an air/carbon dioxide atmosphere (95/5%), the medium was removed and stored at -20°C until assayed for hormone content.

Growth hormone was measured in a conventional radioimmunoassay using anti-growth hormone antibody.

The effect of 9 different peptides on the release of growth hormone in cultured rat pituitary cells is shown in Figs. 1 and 2. The peptides DC-25-4 (Figure 1) and DC-25-24 (Figure 2) are most active in inhibiting the release of growth hormone. Both DC-25-4 and DC-25-24 contain an electron withdrawing group near one end of the molecule and an electron donating group near the opposite end of the molecule. Peptides DC-23-85 (Figure 1) and DC-25-16 (Figure 2), which are not within the present invention, show essentially no activity.

Inhibition of I^{125} Somatotropin-release-inhibiting factor (SRIF-14) binding by linear somatostatin analogs

Crude membrane preparations were obtained from rat pancreas, cerebral cortex, or human small cell lung carcinoma (NCI-H69) cells by homogenizing (Polytron, setting 6, 15 sec) the tissues or cells in ice-cold 50 mM Tris-HCl and centrifuging twice at 39,000 x g (10 min), with an intermediate resuspension in fresh buffer. The final pellets were resuspended in 10 mM Tris-HCl for assay. Aliquots of the membrane preparation were incubated for 25 min at 30°C with labeled somatotropin-release-inhibiting factor, [^{125}I-Tyr^{11}] SRIF-14 (2000 Ci/mmol, Amersham Corp.), in 50 mM HEPES (pH 7.4) containing bovine serum albumin (10 mg/ml;

fraction V, Sigma Chem.), $MgCl_2$ (5mM), Trasylol (200 KIU/ml), bacitracin (0.02 mg/ml), and phenylmethylsulphonyl fluoride (0.02 mg/ml). The final assay volume was 0.3 ml. The incubations were terminated by rapid filtration through Whatman GF/C filters (pre-soaked in 0.3% polyethylenimine) under reduced pressure. Each tube and filter were then washed three times with 5 ml aliquots of ice-cold buffer. Specific binding was defined as the total $[^{125}I]$SRIF-14 bound minus that bound in the presence of 200 nM unlabelled SRIF-14.

Table 1 gives results of inhibition of $[^{125}I]$SRIF-14 binding by linear peptides of the invention. The concentration of $[^{125}I]$SRIF-14 was approximately 0.05 nM. (Values in parenthesis indicate the number of independent determinations.) The IC_{50} (concentration of analog resulting is 50% competitive inhibition) in nM values are indicated for pancreas, small cell lung carcinoma (SCLC), and brain. The results show that analogs DC-25-4 and DC-23-99 are particularly effective in inhibiting the binding of I^{125} SRIF-14. Peptide DC-23-85, which is not within the invention, inhibits the binding of I^{125}SRIF-14 only poorly.

Use

When administered to mammals, particularly humans, (e.g. orally, topically, intravenously, parenterally in a sustained release, biodegradable or nonbiodegradable form, nasally, or by suppository), the compounds can be effective to

inhibit growth hormone release as well as to inhibit somatomedins (e.g., IGF-1), insulin, glucagon, other autoparacrine growth factors or pancreatic exocrine secretion, and to therapeutically affect the central nervous system.

The compounds can be administered to a mammal, e.g. a human, in the dosages used for somatostatin or, because of their greater potency, in smaller dosages. The compounds of the invention can be used for the treatment of cancer, particularly growth hormone-dependent cancer (e.g., bone, cartilage, pancreas (endocrine and exocrine), prostate, or breast), acromegaly and related hypersecretory endocrine states, or of bleeding ulcers in emergency patients and in those suffering from pancreatitis or diarrhea. The compounds can also be used in the management of diabetes and to protect the liver of patients suffering from cirrhosis and hepatitis. The compounds can also be used to treat Alzheimer's disease, as analgesics to treat pain by acting specifically on certain opiate receptors, and as gastric cytoprotective compounds for ulcer therapy. The compounds can also be used to treat certain types of mushroom poisoning.

The compounds can also be used to treat diabetes-related retinopathy. The anti-cancer activity of the compounds may be related to their ability to antagonize cancer-related growth factors such as epidermal growth factor.

The compounds can be administered to a mammal, e.g., a

human, in a dosage of 0.01 to 1000 mcg/kg/day, preferably 0.1 to 100 mcg/kg/day.

Mechanism

The activity of previously described analogs of somatostatin is dependent on the presense of a disulfide linkage between cysteine residues located at or near the ends of the peptide, see, e.g., Coy et al. U.S. Patent No. 4,485,101, hereby incorporated by reference. The disulfide linkage results in a cyclic conformation necessary for activity.

The inclusion of a disulfide linkage is an undesirable feature in these synthetic peptides in that the step favoring synthesis of the disulfide linkage imposes a dramatic decrease in the overall yield of the synthesis. Furthermore, the disulfide linkages are subject to oxidation and thus result in a less stable product.

The instant invention avoids the use of disulfide linkages and their attendant drawbacks. The octapeptides of the instant invention utilize non-covalent interactions between the side chains of critically positioned constituent amino acid residues to confer a hairpin or quasi-cyclic conformation on the peptides.

The side chains and substituted side chains of the amino acid residues of the instant invention are subject to two types of interactions that tend to confer the desired tertiary structure on the peptide. The first type of interaction occurs

when amino acids bearing hydrophobic side chains are located at or near both ends of the peptide. Peptides of this structure exploit the tendency of hydrophobic moieties to avoid contact with polar substances.. Interactions between the hydrophobic groups at each end of the peptide, favored over interactions between these groups and the polar solvents of physiological environments, confer a hairpin or quasi-cyclic configuration on the peptide.

The second type of interaction arises as a result of the interaction of electron-donating and electron-withdrawing moieties of amino acids at opposite ends of the peptide. The invention features peptides in which an amino acid possessing an electron-donating group resides in one end region of the peptide while an amino acid possessing an electron-withdrawing group resides in the other end region of the peptide. The attraction between the electron-donating group, at one end of the peptide, and the electron-withdrawing group, at the other end of the peptide, acts to confer a hairpin or quasi-cyclic structure on the peptide. Both hydrophobic-hydrophobic interactions and electron donor-elctron withdrawer interactions may be active in a given peptide.

Other embodiments are within the following claims.

What is claimed is:

Claims

1. An octapeptide of the formula

$$\begin{array}{l} R_1 \diagdown \\ \qquad A^1 - A^2 - A^3 - \text{D-Trp} - \text{Lys} - \\ R_2 \diagup \qquad\quad A^6 - A^7 - A^8 - R_3 \end{array}$$

wherein

A^1 is a D-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, NO_2), p-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

A^2 is any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, or NO_2), p-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, or NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

A^3 is any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, Trp, Tyr, ß-Nal, o-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, or NO_2), p-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, or NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

A^6 is any of Ala, pyridyl-Ala, Leu, Ile, Val, Lys, Met, Nle, Thr-R_4, Trp, Ser-R_4, ß-Nal, o-X-Phe (wherein X =

CH_3, Cl, Br, F, OH, OCH_3, or NO_2), p-X-Phe (wherein X = CH_3, Cl, Br, F, OH, OCH_3, or NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

A^7 is any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, or NO_2), p-X-Phe (wherein X = H, CH_3, Cl, Br, F, OH, OCH_3, or NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

A^8 is a D- or L-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Ser-R_4, Thr-R_4, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = CH_3, Cl, Br, F, OH, OCH_3, or NO_2), p-X-Phe (wherein X = CH_3, Cl, Br, F, OH, OCH_3, or NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe;

each R_1 and R_2, independently, is any of H, lower acyl, or lower alkyl; and R_3 is H, NH_2, or lower alkyl; provided that at least one of A^1 and A^8 must be an aromatic amino acid; and further provided that if either A^2 or A^7 is an aromatic amino acid, then A^8 cannot be an aromatic amino acid; and further provided that R_4 may be nothing or may be a carbohydrate, e.g., $C_x(H_2O)_y$, where x is 1-18 and y is 1-16, linked through the hydroxyl group of Ser or Thr; or a pharmaceutically acceptable salt thereof.

2. The octapeptide of claim 1, wherein one but not both of A^1 and A^2 are aromatic amino acids; and wherein one but not both of A^7 and A^8 are aromatic amino acids.

3. The octapeptide of claim 1, wherein A^1 is a D-isomer of any of Trp, ß-Nal, o-X-Phe (wherein X = CH_3 or OCH_3), p-X-Phe (wherein X = CH_3 or OCH_3) and A^8 is a D- or L-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Ser, Thr, Val, Met, Nle, o-X-Phe (wherein X = Cl, Br, F, OH, or NO_2), p-X-Phe (wherein X = Cl, Br, F, OH, or NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe.

4. The octapeptide of claim 1, wherein A^1 is a D-isomer of any of o-X-Phe (wherein X = H, Cl, Br, F, OH, NO_2), p-X-Phe (wherein X = H, Cl, Br, F, OH, NO_2), 2,4-dichloro-Phe, pentafluoro-Phe, or L-Phe and A^8 is a D- or L-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Thr, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = CH_3 or OCH_3), or p-X-Phe (wherein X = CH_3 or OCH_3).

5. The octapeptide of claim 1, wherein A^8 is a D- or L-isomer of any of Thr, Trp, ß-Nal, o-X-Phe (wherein X = CH_3 or OCH_3), or p-X-Phe (wherein X = CH_3 or OCh_3) and A_1 is a D-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, o-X-Phe (wherein X = H, Cl, Br, F, OH, NO_2), p-X-Phe (wherein x = H, Cl, Br, F, OH, NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe.

6. The octapeptide of claim 1, wherein A^8 is a D- or L-isomer of any of Ser, Thr, o-X-Phe (wherein X = Cl, Br, F, OH, NO_2), p-X-Phe (wherein X = Cl, Br, F, OH, NO_2), 2,4-dichloro-Phe, or pentafluoro-Phe and A^1 is a D-isomer of any of Ala, pyridyl-Ala, Leu, Ile, Val, Met, Nle, Trp, ß-Nal, o-X-Phe (wherein X = CH_3 or OCh_3), or p-X-Phe (wherein X = CH_3 or OCH_3).

7. The octapeptide of claim 1, wherein
A^1 = ß-D-Nal or D-Phe;
A^2 = Ala, Phe or p-chloro-Phe;
A^3 = Tyr or Phe;
A^6 = Val, Lys, or Thr;
A^7 = Ala or Phe; and
A^8 = Thr or ß-D-Nal.

8. The octapeptide of claim 2, of the formula:
D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2;
D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;
D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2; or
D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-ß-D-Nal-NH_2.

9. A therapeutic composition capable of inhibiting the release of growth hormone, somatomedins (e.g., IGF-1), insulin, glucagon, other autoparacrine growth factors, or pancreatic exocrine secretion comprising a therapeutically effective amount of the compound of claim 1 together with a pharmaceutically acceptable carrier substance.

10. A method of treating a mammal in need of reduction of growth hormone, insulin, somatomedins (e.g., IGF-1), glucagon, other autoparacrine growth factors, or pancreatic exocrine secretion comprising administering to said mammal a therapeutically effective amount of the compound of claim 1.

11. The therapeutic composition of claim 9 wherein said composition is in the form of a pill, tablet, or capsule for oral administration to a human patient in need of said compound.

12. The therapeutic composition of claim 9 wherein said composition is in the form of a liquid for oral administration to a human patient in need of said compound.

13. The therapeutic composition of claim 11, said composition being coated with a substance capable of protecting said composition from the gastric acid in the stomach of said human patient for a period of time sufficient to allow said composition to pass undisintegrated into the small intestine of said human patient.

14. The therapeutic composition of claim 9, said composition being in the form of a cream, gel, spray, or ointment applied topically or iontophoretically forced through the skin of a human patient in need of said compound.

15. The therapeutic composition of claim 9, said composition being in the form of a liquid capable of being administered nasally as drops or spray to a human patient in need of said compound.

16. The therapeutic composition of claim 9, said composition being in the form of a liquid for intravenous, subcutaneous, parenteral, or intraperitoneal administration to a human patient in need of said compound.

17. The therapeutic composition of claim 9, said composition being in the form of a biodegradable or nonbiodegradable sustained release composition for intramuscular administration to a human patient in need of said compound.

Table I

Table 1

Inhibition of I^{125} SRIF-14 binding by linear analogs of somatostatin

	IC_{50} (nM)		
Analog	Pancreas	SCLC	Brain
Somatostatin	0.53 (5)	4.2 (5)	0.53 (3)
BIM-23053/DC-25-4	2.8 (2)	2.2 (1)	109
BIM-23052/DC-23-99	9.4 (1)	1.2 (1)	7.3 (1)
BIM-23049/DC-23-76	9.2 (3)	2.1 (1)	>10,000 (1)
BIM-23051/DC-23-89	34 (2)	15 (1)	>10,000 (1)
BIM-23050/DC-23-85	264 (1)	---	2,189 (2)

Results are expressed as the concentration in nM of analog that gives 50% inhibition of I^{125} SRIF-14 binding (IC_{50}). The numbers in parantheses indicate the number of trials. The structure of the analogs is as follows: BIM-23049/DC-23-76--ß-D-Nal-Ala-Tyr-D-Trp-Lys-Val-Ala-Thr-Nh_2; BIM-23050/DC-23-85--n-methyl-D-Ala-Tyr-D-Trp-Lys-Val-Phe-NH_2; BIM-23051/DC-23-89--D-Phe-Ala-Phe-D-Trp-Lys-Thr-Ala-Thr-NH_2; BIM-23052/DC-23-99--D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2; BIM-23053/DC-25-4--D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-ß-D-Nal-NH_2. The structure of SRIF-14 is: Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Ser-OH.

FIG. 1

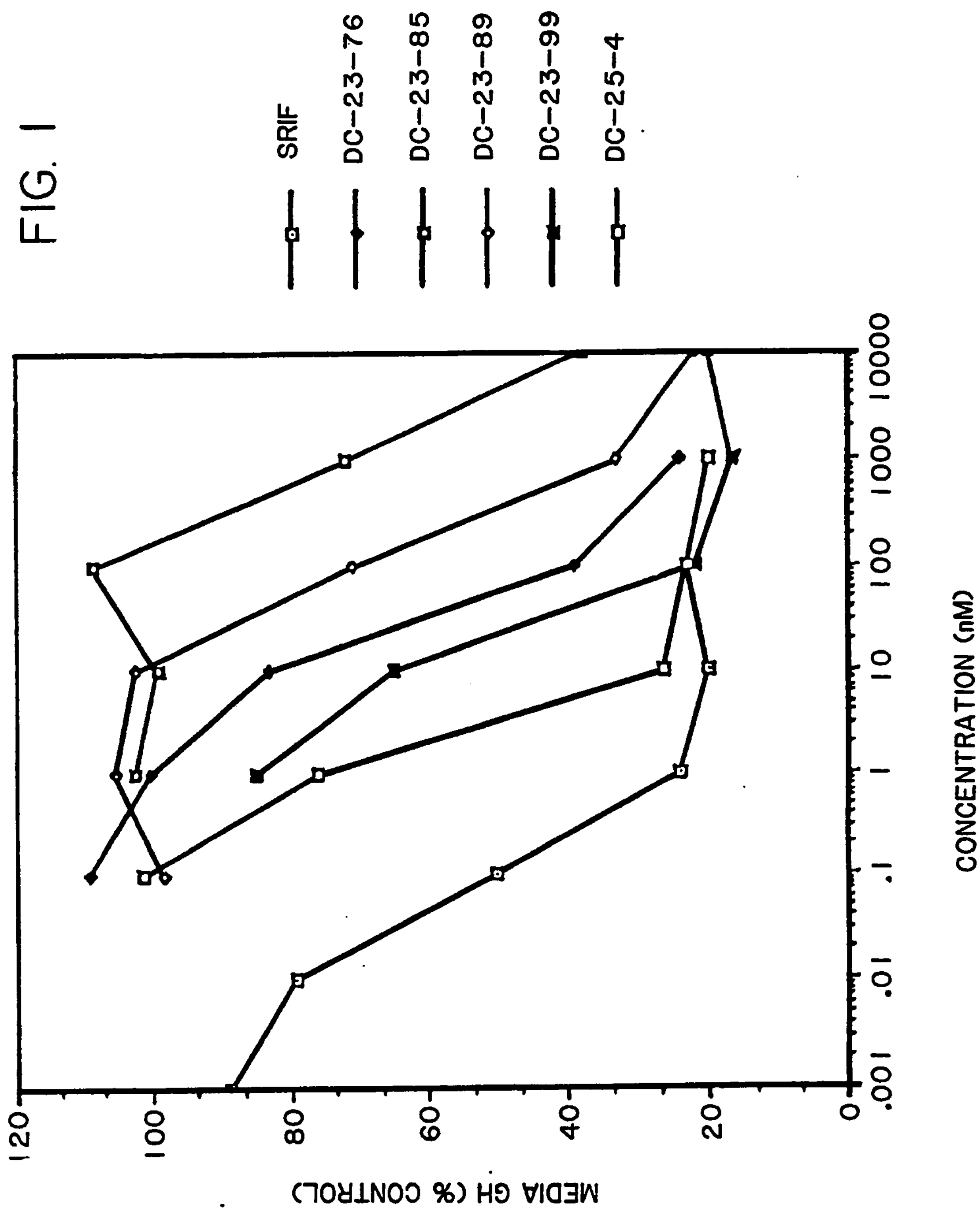
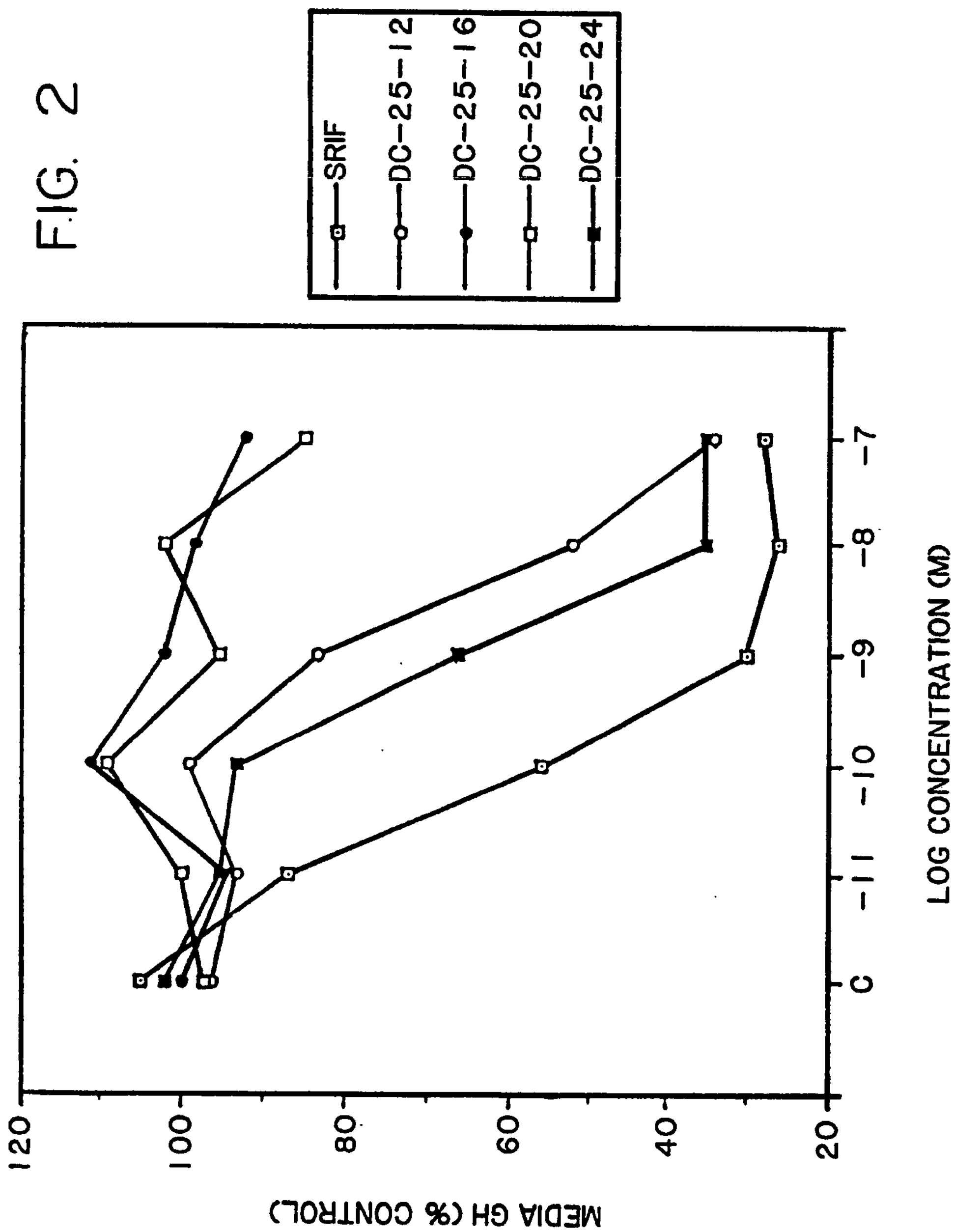

SRIF
DC-23-76
DC-23-85
DC-23-89
DC-23-99
DC-25-4
MEDIA GH (% CONTROL)
120
100
80
60
40
20
0
CONCENTRATION (nM)
.001
.01
.1
1
10
100
1000
10000

FIG. 2


SRIF
DC-25-12
DC-25-16
DC-25-20
DC-25-24
MEDIA GH (% CONTROL)
120
100
80
60
40
20
C
-11
-10
-9
-8
-7
LOG CONCENTRATION (M)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/02196

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C07K 7/26; A61K 37/02
U.S. CL.: 530/311, 328; 514/16, 806

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System [5]	Classification Symbols
U.S.	530/311, 328; 514/16,806

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US, A, 4,185,010 (SARANTAKIS), 22 JANUARY 1980, See the entire document.	1,2,4,5 and 8-17
A	US, A, 4,238,481 (RINK ET AL.), 09 December 1980, See the entire document.	1,2,4,5 and 8-17
A	US, A, 4,728,638 (BAUER ET AL.), 01 March 1988, See the entire document.	1,2,4,5 and 8-17
P,A	US, A, 4,871,717 (COY ET AL.), 03 October 1989, See the entire document.	1,2,4,5 and 8-17
A	US, A, 4,797,469 (DIAZ ET AL.), 10 January 1989, See the entire document.	1,2,4,5 and 8-17
Y	CHEMICAL ABSTRACTS, Vol. 109, No. 23, 1988 (Columbus, Ohio, USA) D. Coy, "Preparation of somatostatin analogs as drugs", see page 711, column 1, abstract No. 211493V, Eur. Pat. App. 277,419, 10 August 1988.	1,2,4,5 and 8-17
Y	CHEMICAL ABSTRACTS, Vol. 110, No. 25, 1989 (Columbus, Ohio, USA) D. Coy, "Preparation of Therapeutic somatostatin analogs", See page 698, columns 1 and 2, abstract No. 232105f, Eur. Pat. App. 298,732, 11 January 1989.	1,2,4,5, and 8-17

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
16 JULY 1990	13 AUG 1990
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	for T. D. WESSENDORF Nguyen Nguyen NGUYEN NGOC-HO INTERNATIONAL DIVISION

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	CHEMICAL ABSTRACTS, Vol. 83, No. 9, 1975 (Columbus, Ohio, USA) H. Leblanc, "Comparison of cyclic and linear forms of somatostatin in the inhibition of growth hormone, insulin and glucagon secretion", see page 143, column 1, abstract No. 72539u, J. Clin Endocrinology Metab., 1975, 40(5), 906-8 (Eng.).	1,2,4,5 and 8-17

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter[1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out[1], specifically:

3. ☐ Claim numbers__________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

MULTIPLE SPECIES

(SEE ATTACHMENT)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

   Claim 8, last specie (and claim 1, first appearing specie).
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.